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(54) Title: COMPOUNDS USEFUL TO MIMIC PEPTIDE BETA-STRANDS

(57) Abstract: The present invention relates generally to new compositions of matter and methods of using such compositions of matter for investigational or therapeutic purposes, and more particularly to compositions that a) mimic β-strands, b) block β-sheet dimerization of proteins, c) block protein-protein β-sheet interactions and/or interact with proteins by β-sheet formation and as well as d) methods for investigating or designing compounds that mimic β-strands and/or β-sheets, e) methods for treating diseases or disorders by administering therapeutically effective amounts of such compounds to human or veterinary patients and f) methods for using a new protecting group (i.e, 2,7-di-*tert*-butylfluorenylmethoxycarbonyl(Fmoc*)) in peptide syntheses.

COMPOUNDS USEFUL TO MIMIC PEPTIDE BETA-STRANDS

Related Applications

This application claims priority to copending United States Provisional Patent Application Nos. 60/150,260 filed on August 23, 1999, 60/151,161 filed August 27, 1999 and 60/152,696 filed September 7, 1999, the entireties of which are expressly incorporated herein by reference.

Field of the Invention

The present invention relates generally to new compositions of matter and methods of using such compositions of matter for investigational or therapeutic purposes, and more particularly to compositions that a) mimic β-strands, b) block β-sheet dimerization of proteins, c) block protein-protein β-sheet interactions and/or interact with proteins by β-sheet formation and as well as d) methods for investigating or designing compounds that mimic -strands and/or β-sheets, e) methods for treating diseases or disorders by administering therapeutically effective amounts of such compounds to human or veterinary patients and f) methods for using a new protecting group (i.e., 2,7-di-*tert*-butylfluorenylmethoxycarbonyl (Fmoc*)) in peptide syntheses.

Background of the Invention

Recognition between exposed edges of β-sheets is an important mode of protein-protein interaction. *See,* "β-Sheet Interactions Between Proteins", Maitra, S.; Nowick, J. S. In: *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*; Greenberg, A.; Breneman C. M.; Liebman, J. F., Eds.; Wiley: New York, 2000; Chapter 15. These β-sheet interactions between proteins are, for example, critical in the binding of neuronal nitric oxide synthase inhibitory protein to neuronal nitric oxide synthase. Liang, J.; Jaffrey, S. R.; Guo, W.; Snyder, S. H.; Clardy, J. *Nat. Struct. Biol.* 1999, *6*, 735-740. Also, β-sheet interactions between proteins are involved in the binding

of PDZ domains to membrane receptor and ion channel proteins. Doyle, D. A.; Lee, A.; Lewis, J.; Kim, E.; Sheng, M.; MacKinnon, R. *Cell* 1996, *85*, 1067-1076. Additionally, β-sheet interactions between proteins play a role in the binding of Ras oncoproteins to the Raf kinase, an example of which is illustrated in Figure 1.

Interactions between the edges of β-strands and/or β-sheets are also involved in protein dimerization and in peptide and protein aggregation, wherein the edges of protein β-sheets provide alternating arrays of hydrogen-bond donors and acceptors, in the pattern –donor-acceptor, donor-acceptor, donor-acceptor, etc. In β-sheet interactions between proteins, the edges of β-strands and/or β-sheets hydrogen bond together. Chemical decoys that duplicate the hydrogen-bonding of edges of protein β-strands and/or β-sheets hold promise as inhibitors of protein-protein interactions. Although this promise has not yet been achieved, it has been pursued by several researchers within the past decade. For example, Michne and Schroeder have created a bicyclic compound that mimics the hydrogen-bonding pattern of one edge of a peptide β-strand to block a putative β-sheet interaction between lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). Michne, W. F.; Schroeder, J. D. *Int. J. Pept. Protein Res.* 1996, *47*, 2-8. Also, Rebek, Pallai, and coworkers have developed bi- and tricyclic β-strand mimics to inhibit a postulated β-sheet interaction between gp120 and the CD4 receptor. Roberts, J. C.; Pallai, P. V.; Rebek, J., Jr. *Tetrahedron Lett.* 1995, *36*, 691-694; Boumendjel, A.; Roberts, J. C.; Hu, E.; Pallai, P. V.; Rebek, J., Jr. *J. Org. Chem.* 1996, *61*, 4434-4438. Related studies by Schrader and Kirsten have focused on the development of peptidomimetic compounds that mimic the hydrogen-bonding pattern of one edge of a peptide β-strand and bind peptides through β-sheet interactions. Schrader, T.; Kirsten, C. *Chem. Commun.* 1996, 2089-2090; Kirsten, C. N.; Schrader, T. H. *J. Am. Chem. Soc.* 1997, *119*, 12061-12068.

Both hydrogen bonding and other non-covalent interactions contribute to β-sheet interactions between proteins. Generally, the hydrogen-bonding interactions between the edges of protein β-sheets are accompanied by additional polar and hydrophobic interactions between the amino acid side

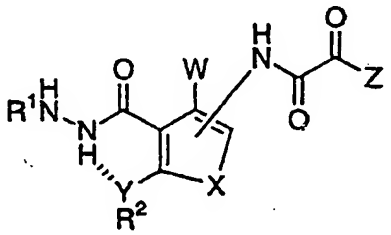
chains. Contacts with other structures, such as helices, can also be present. Collectively, these important interactions provide strength and specificity to the protein-protein interactions. A variety of systems that mimic and block these interactions have been developed. *see, for example,* Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P., Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* 1994, *116*, 9947-9962; Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Guzman, M. C.; Yokoyama, A.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Schleif, W. A. *J. Am. Chem. Soc.* 1995, *117*, 11113-11123; Smith, A. B., III; Benowitz, A. B.; Sprengeler, P. A.; Barbosa, J.; Guzman, M. C.; Hirschmann, R.; Schweiger, E. J.; Bolin, D.R.; Nagy, Z.; Campbell, R. M.; Cox, D. C.; Olson, G. L. *J. Am. Chem. Soc.* 1999, *121*, 9286-9298.

Compounds that mimic the structures and hydrogen-bonding patterns of protein β-sheets but that have not specifically been targeted toward binding proteins have also been reported. Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* 1996, *25*, 401-415. In studies conducted during the late 1980s, Kemp and coworkers described a 2,8-diaminoepindolidione molecular template that mimics the hydrogen-bonding functionality of one edge of a peptide β-strand and have coupled this β-strand mimic to peptides to generate intramolecularly hydrogen-bonded β-sheetlike structure. Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* 1988, *29*, 5077-5080; Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* 1988, *29*, 5081-5082; Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* 1990, *55*, 4650-4657

Given the variety of biological and physiological functions that are mediated or affected by β-sheet interactions between proteins, it is apparent that compounds or methods that can either mimic or block such β-sheet interactions may be useable for various therapeutic or medical applications. Accordingly, there exists a need in the art for the development of new compositions and/or methods that mimic the actions of tripeptide β-strands, block β-sheet dimerization of proteins, block protein-protein β-sheet interactions and/or interact with proteins by β-sheet interaction

Summary of the Invention

The present invention provides new compositions of matter that mimic β-strands and, therefore, affect β-sheet interactions of proteins. These compositions have the general Formula A:



wherein;

R^1 = H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

R^2 = H, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

W = H, F or the NH-CO-CO-Z group shown,

X = O, S, NR^3, CR^4=N, N=CR^4, CR^4 =CR^5;

R^3 is H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

R^4 and R^5 are each selected from H, alkyl, halogen, nitro, carboxyl, amino, alkyl or aryl sulfone, alkyl or aryl sulfoxide, sulfonic acid, sulfonate salt or sulfonamide, and wherein R^4 and R^5 may be combined to form a ring structure;

Y = O, S, or YR^2 as a group may be a halogen;

Z = OR^6 or NR^7R^8, wherein R^6, R^7 and R^8 are each selected from H, acyl, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide.

In accordance with the present invention, pharmaceutically acceptable preparations of the foregoing compounds may be administered to mammalian patients or introduced into in vitro systems.

Further in accordance with the present invention, the foregoing compounds of this invention may be incorporated into peptides, peptidomimetics, or proteins to cause such peptides, peptidomimetics, or proteins to dimerize by means of β-sheet interactions.

Still further in accordance with the present invention, one or more of the forgoing compounds may be used as, combined with or incorporated into a drug or agent that is intended to mimic β-strands in vivo or in vitro.

Still further in accordance with the present invention, one or more of the forgoing compounds may be used as, combined with, or incorporated into a drug or agent that is intended to block the β-sheet dimerization of proteins, in vivo or in vitro.

Still further in accordance with the present invention, one or more of the forgoing compounds may be used as, combined with or incorporated into a drug or agent that is intended to block protein-protein β-sheet interactions, in vivo or in vitro.

Still further in accordance with the present invention, one or more of the forgoing compounds may be used as, combined with or incorporated into a drug or agent that is intended to interact with a protein by β-sheet formation, in vivo or in vitro.

Still further in accordance with the present invention, there is provided a new protecting group, 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl (referred to herein as Fmoc* and shown in Figure 12). This new protecting group is usable to solublize or facilitate the reaction of compounds of Formula A above. Also, in any peptide syntheses where amino acids or amino acid surrogates such as Hao are coupled sequentially to form a growing peptide chain or designed peptidomimetic molecule, the Fmoc* group may be used to protect the *N* terminus of each amino acid (or amino acid surrogate such as Hao) as it is added to the peptide chain. In this regard, Fmoc* may be added to the *N* terminus of an amino acid to form an Fmoc*-protected amino acid. The Fmoc*-

protected amino acid may then be linked by a peptide linkage to another amino acid to form a dipeptide or to an existing peptide chain to form a polypeptide, such that the Fmoc* then becomes located at the *N*- terminal end of the peptide chain. That Fmoc* group can then the removed from the end of the peptide chain and another Fmoc*-blocked amino acid may then be linked to the chain by way of a peptide linkage, such that the Fmoc* group of that Fmoc*-protected amino acid becomes located on the end of the peptide chain. This process is repeated until the desired peptide or peptide like molecule has been formed.

Brief Description of the Drawings

Figure 1 is a Molscript™ diagram of the complex between the Ras-binding domain of the c-Raf1 kinase (upper) and the Ras analogue, Rap1A (lower).

Figure 2 shows the combination of Hao and tripeptides to generate compounds that mimic β-sheets and form well-defined β-sheet dimers.

Figure 3 illustrates the chemical structure of Hao and shows its relationship to *a* tripeptide.

Figure 4 shows a method by which Hao is prepared as its 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl protected derivative 8 by the condensation of suitably protected derivatives of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid.

Figure 5 shows a scheme by which one may use these Hao derivatives to prepare the tripeptide *i*-PrCO-Phe-Hao-Val-NHBu. (Tripeptide A)

Figure 6 shows the structure of a sheetlike hydrogen-bonded dimer derived from Hao in $CDCl_3$ solution whereon interstrand NOEs are illustrated by arrows.

Figure 7 is graphic representation of ^1H NMR data points wherein concentration in $CDCl_3$ solution at 295 K is on the x axis and the chemical shift of α-protons of 9 is on the y axis and wherein the curves represent dimerization isotherms that best fit the data points.

Figure 8 shows two different conformations of Hao, in which Hao is part of a larger molecule, namely *i*-PrCO-Hao-NHMe.

Figure 9 shows the chemical structure of *i*-PrCO-Phe-Hao-Val-NHBu

(Tripeptide A).

Figure 10 shows a Molecular model of Ac-Ala-Hao-Ala-NHMe docked to the β-sheet edge of the Ras analogue, Rap1A.

Figure 11 shows an unfolded Hao-containing peptide folding into an antiparallel β-sheet or, alternatively, a parallel β-sheet.

Figure 12 shows a comparison of the chemical structures of Fmoc, a protecting group used in prior art peptide syntheses, and Fmoc* a protecting group used in synthesizing peptides and other peptide-like compounds in accordance with this invention.

Detailed Description of Exemplary Embodiments

Compounds of the present invention having the above-set-forth general formula have been determined by applicant to mimic the action of β-strands (referred to herein as "β-strand mimics"). These β-strand mimics may be based on aminoaromatic derivatives and may be combined with urea-based turn units and peptide strands to create a variety of β-sheetlike structures.

For example, Figure 2 shows an example of the manner in which Hao may be combined with tripeptides to generate compounds that mimic β-sheets and form well-defined β-sheet dimers. In the particular example of Figure 2, which is the 5-amino-2-methoxybenzoic acid molecule, hydrazide and oxalamide groups within the molecules can, in some respects, be viewed collectively as an unnatural amino acid that duplicates the hydrogen-bonding functionality of one edge of a tripeptide β-strand. This amino acid comprises hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid groups and is designated herebelow, in at least some instances, by the three-letter abbreviation "Hao" to reflect its three components. Figure 3 illustrates the chemical structure of Hao and shows its relationship to a tripeptide. Hao is one example of a composition of matter in accordance with this invention.

The following paragraphs contain a detailed discussion of the properties of Hao, a method for preparing its *N*-protected derivatives, the use of these *N*-protected derivatives in peptide synthesis, and the propensity of an Hao-containing peptide to form a β-sheetlike dimer.

As shown in Figure 4, Hao is readily prepared as its 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl protected derivative 8 by the condensation of suitably protected derivatives of hydrazine, 5-amino-2-methoxybenzoic acid, and oxalic acid. The 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl (Fmoc*) group is used in place of the popular fluorenylmethyloxycarbonyl (Fmoc) group to improve the solubility of Hao and its precursors in organic solvents. Reaction of 2,7-di-*tert*-butylfluorenylmethyloxycarbonyl chloride (Fmoc*-Cl) with anhydrous hydrazine affords Fmoc*-hydrazine 3. Coupling with 2-methoxy-5-nitrobenzoyl chloride 4 gives hydrazide 5. Reduction of the nitro group generates amine 6. Condensation with ethyl oxalyl chloride gives amide 7. Hydrolysis of the ethyl ester group with NaOH, followed by passage of the reaction mixture through acidic ion exchage resin, yields Fmoc*-Hao 8. When this procedure is performed on a multigram scale, a 75% yield for the conversion of Fmoc*-Cl to Fmoc*-Hao is obtained. The Boc-protected derivative of Hao (Boc-Hao) is prepared in a similar fashion from Boc-hydrazine.

With reference to Figure 5, in order to evaluate the synthetic and structural properties of Hao, one may use these Hao derivatives to prepare the tripeptide *i*-PrCO-Phe-Hao-Val-NHBu, the structure of which is shown in Figure 9 and which is referred to herebelow as "Tripeptide A". Tripeptide A, which is another exemplary composition of this invention, may be prepared by different methods. Tripeptide A is a simple, non-functionalized peptide derivative that is suitable for ^{1}H NMR studies in chloroform solution.

In one preparation method, Tripeptide A may be prepared manually on poly(ethylene glycol)–polystyrene (PEG-PS) resin with a tris(alkoxy)benzylamide (PAL) linker using a modified version of the previously reported Backbone Amide Linker (BAL) strategy. In our modification of the previously reported BAL strategy, we introduce the butyl group by converting the PAL amino group to its *o*-nitrobenzenesulfonamide by treatment with *o*-nitrobenzenesulfonyl chloride (NsCl), alkylation with *n*-butyl iodide and 1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (MTBD), and removal of the nitrobenzenesulfonamide group with mercaptoethanol and DBU. Coupling of Fmoc-valine to the resulting sterically hindered (Bu)PAL-PEG-PS proves difficult using standard coupling

reagents, but proceeds smoothly using the symmetrical anhydride in CH_2Cl_2-DMF (8:2). The Hao and Phe residues are introduced by double coupling with DCC and HOBt; the isobutryic acid residue is introduced by single coupling under similar conditions. Cleavage from the resin with TFA then affords Tripeptide A. This process is shown in Figure 5.

Indole resin (commercially available as, PS-Indole-CHO, Argonaut Technologies, San Carlos, California) may be used in preparing Tripeptide A. Indole resin bears an indole-3-carboxaldehyde linker, which allows the butylamine portion of the tripeptide to be introduced by reductive amination. Subsequent introduction of the amino acid residues with DCC and HOBt proceeds smoothly. The product produced by this route is slightly more pure than that generated on the PEG-PS resin with the PAL linker. For this reason, and because of the absence of the need of the symmetrical anhydride, we consider this linker superior and have adopted it for subsequent solid-phase syntheses of related *N*-alkylamides.

The Tripeptide A is synthesized in solution by sequential coupling of Val-NHBu, Boc-Hao, Boc-Phe, and isobutyric acid using 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and HOBt in a mixture of THF and DMF. This synthesis proceeds smoothly, although the limited solubility of the Hao-containing intermediates may necessitate using DMF as a co-solvent. Thus, it is apparent that Fmoc*-Hao and Boc-Hao behave like regular amino acids in both solid-phase and solution-phase peptide syntheses with carbodiimide coupling agents.

To evaluate the effect of Hao upon peptide structure, the Tripeptide A is studied by ^{1}H NMR spectroscopy. ^{1}H NMR chemical shift, NOE, and dilution titration studies indicate that this tripeptide forms a β-sheetlike hydrogen-bonded dimer in $CDCl_3$ solution. Figure 6 illustrates the structure of this dimer. As shown in Figure 6, NH protons H_b, H_c, H_d, and H_f are hydrogen bonded, while NH protons H_a and H_e are not. In the ^{1}H NMR spectrum in $CDCl_3$ solution, hydrogen-bonded NH protons typically appear about 2 ppm downfield of non-hydrogen-bonded NH protons. Peptide amides generally appear at ca. 6 ppm when not hydrogen bonded and at ca. 8 ppm when hydrogen bonded. In Tripeptide A, the

chemical shift of the (non-hydrogen-bonded) phenyalanine NH_a group is 6.35 ppm in 7 mM $CDCl_3$ solution at 295 K, while that of the (hydrogen-bonded) valine butylamide NH_f group is 7.93 ppm. The three NH protons of Hao, H_b, H_c, and H_d appear at 11.74, 11.18, and 10.71 ppm, respectively. These values are comparable to those of the corresponding hydrogen-bonded protons in compounds that mimic β-sheets 1a and b (11.3, 11.2, and 10.7 ppm, respectively). The remaining NH proton (H_e) appears at 8.41 ppm. This proton belongs to an oxalamide system and appears significantly downfield of that of the NH proton of *N,N'*-dibutyloxalamide in dilute $CDCl_3$ solution (7.44 ppm, 7 mM), conditions at which it is not hydrogen bonded. The downfield shifting of this proton relative to the dibutyloxalamide control appears too small to arise from a typical hydrogen bond, and may instead result from magnetic anisotropy associated with the valine carbonyl group and Hao aromatic ring.

The chemical shifts of the amino acid α-protons of Tripeptide A are also consistent with the dimeric β-sheetlike structure shown in Figure 6. Protein α-protons generally resonate downfield of those in random coils by several tenths of a ppm in β-sheets. In Tripeptide A, the Phe and Val α-protons appear at 5.38 and 4.62 ppm respectively, substantially downfield of the corresponding random coil values (4.66 and 3.95 ppm). Also consistent with the dimeric structure, the chemical shift of the Phe α-proton in Tripeptide A is comparable to the limiting chemical shift of the Phe α-proton in the dimer of compounds that mimic β-sheet 1a (5.29 ppm).

The presence of interstrand NOES is a hallmark of β-sheets. ^1H NMR transverse-ROESY (Tr-ROESY) studies in $CDCl_3$ solution (7 mM, 30 °C) show that Tripeptide A exhibits strong NOEs between the Phe and Val α-protons and weaker NOEs between the Val γ (methyl) protons and the Phe α, β, and δ protons. Figure 6 illustrates these NOEs with arrows. These long-range NOEs can not easily be explained by intramolecular contacts, but are wholly consistent with the dimeric structure.

Intrastrand NOE data also provide evidence that Tripeptide A adopts a β-strandlike conformation. Notably, the aromatic amino proton (H_d) exhibits a strong NOE with the aromatic H_6 proton but exhibits no NOE with the aromatic

H_4 proton. Similarly, the Val α-proton exhibits a stronger interresidue NOE with butylamide proton H_f and a weaker NOE with the Val NH proton (H_e). When a 300 ms mixing time is used in the Tr-ROESY experiment, the Phe α-proton exhibits weak inter- and intraresidue NOEs with both the Hao NH proton H_b and the Phe NH proton (H_a). The weakness of the interresidue NOE appears to result from an unusually short transverse relaxation time (and associated $T_{1\rho}$) for the Hao NH proton H_b. The short relaxation time of this proton, evidenced by the broadness of its peak in the ^1H NMR spectrum, should result in loss of phase coherence during the mixing period of the Tr-ROESY experiment and generate an anomalously weak NOE. Consistent with this explanation, the Phe α-proton gives a relatively strong interresidue NOE with the Hao NH proton H_b and little or no intraresidue NOE with the Phe NH proton (H_a) when a shorter (100 ms) mixing time is used. Coupling constant data provide further evidence for a β-strandlike conformation, with $^3J_{HN\alpha}$ values of 8.4 Hz (Phe) and 9.6 Hz (Val).

Tripeptide A is too strongly dimerized in pure $CDCl_3$ to allow its dimerization constant to be accurately determined by ^1H NMR dilution titration studies. The NH groups H_a, H_b, and H_c of Tripeptide A exhibit very little concentration dependence in chemical shift (0.05-0.07 ppm from 0.16 to 2.6 mM) in $CDCl_3$ solution and show saturation at higher concentrations. These data indicate that Tripeptide A is virtually completely dimerized at NMR accessible concentrations. Analysis of this very limited titration data set reveals a dimerization constant of ca. 10^6 M^{-1}. This value is dramatically larger than that of the tripeptide *i*-PrCO-Phe-Leu-Val-NHBu, which is prepared as a control and found to have a dimerization constant of 100 – 200 M^{-1} by fitting dimerization isotherms to the NH and α-proton chemical shifts at varying concentrations. Several pentapeptides are also prepared as controls but are found to be too insoluble for ^1H NMR titration studies.

Addition of the competitive solvent CD_3OD to the $CDCl_3$ weakens the dimerization of Tripeptide A, thereby allowing a dimerization constant to be determined by ^1H NMR dilution titration studies. Thus, dilution titration studies, in which the chemical shifts of the α-protons are monitored as a function of concentration and a dimerization isotherm is fitted to the shift data, reveal a

dimerization constant (K_{dim}) of 900 M^{-1} in 10% CD_3OD-$CDCl_3$. Figure 7 illustrates these data and the fitted isotherms. In this solvent system, the control tripeptide *i*-PrCO-Phe-Leu-Val-NHBu self-associates too weakly to allow accurate determination of its dimerization constant by ^1H NMR titration. Analysis of the limited data sets available from ^1H NMR dilution titration reveals a dimerization constant of ca. 5 M^{-1}.

Although Hao is more conformationally constrained than a peptide, it can adopt a conformation that is not β-strandlike as well as one that is β-strandlike. Also, Tripeptide A differs from many if not all of the other systems known to dimerize strongly in that its key structural unit is an unnatural amino acid (i.e., Hao) that can be combined with natural amino acids to give hybrid peptides that are complementary to protein β-sheets. This unnatural amino acid, Hao, imparts a β-sheetlike conformation to peptides that contain it and facilitates their dimerization through β-sheet interactions. In Tripeptide A, Hao provides a β-strandlike edge with an alternating array of hydrogen-bond donors and acceptors that is preorganized to dimerize. Unlike peptides composed solely of natural amino acids, Hao-containing peptides do not readily form higher oligomers, because the aromatic ring of Hao blocks the other edge of the peptide strand.

Compounds that mimic β-sheets 1 can also be viewed as hybrid peptides, consisting of the tripeptide mimic Hao, the turn forming the following dipeptide replacement, -N(Ph)CH_2CH_2N(CH_2CH_2CN)CO-, and a tripeptide region. When written as a peptide, the structure of 1a is:

***i*-PrCO-Hao-N(Ph)CH_2CH_2N(CH_2CH_2CN)CO-Phe-Ile-Leu-NHMe**

In compounds that mimic β-sheets, Hao serves as a template that organizes the tripeptide region into a β-sheet that can dimerize. Thus, Hao imparts both structure (e.g.. conformation and folding) and function (e.g.. dimerization) to peptides in which it is contained.

Specifically, in cases where a β-sheet is formed by folding, such folding may occur in the manner shown in Figure 11. As shown, a molecule that contains Hao (or some other compound of this invention having the general

Formula A above) forms a series of hydrogen bonds with a portion of an attached peptide to form a β-sheet. In that series of hydrogen bonds, the hydrogen-bond donor and acceptor groups of the Hao (or other compound of this invention having the general Formula A above) pair with the hydrogen-bond donor and acceptor groups of the peptide, thereby forming an alternating series of 10-membered and 14-membered rings. In the case of an antiparallel β-sheet, a 10-membered ring, 14-membered ring and 10-membered ring are formed such that the two donors and two acceptors of the Hao-containing molecule are hydrogen bonded. Alternatively in the parallel β-sheet, the donor and acceptor groups of the Hao, or the other embodiments of this invention, form a series of 12-membered hydrogen bonded rings with the amino acid.

Peptides containing Hao display hydrogen-bonding surfaces that are complementary to the hydrogen-bonding edges of protein β-sheets. Figure 10 illustrates this complementarity through a model of Hao-containing peptide Ac-Ala-Hao-Ala-NHMe docked to the β-sheet edge of the Ras analogue, Rap1A. The edge of this simple tripeptide provides an alternating pattern of hydrogen-bond donors and acceptors that matches that of the protein.

Examples

Compounds that mimic β-strands and/or β-sheets may be used to mimic and/or modulate β-sheet interactions in biological systems to provide therapeutic effects or for investigational purposes. The following examples illustrate how compounds that mimic β-sheets may be used.

EXAMPLE 1: Treatments of Cancer

Ras oncoproteins act as molecular switches that activate the serine/threonine kinase c-Raf1 (Raf) by binding to its Ras-binding domain (RBD). This activation is part of the cell signaling pathway that leads to cell growth and plays a key role in cancer. The Ras-related protein Rap1 (Rap) is very similar in structure to Ras and has an identical effector region, but is not membrane bound. Rap also binds to the RBD of Raf, acting as an antagonist, rather than an agonist. X-ray crystallographic structure of the complex between Raf and

Rap shows that the interaction between these proteins involves an antiparallel β-sheet with a rich array of interactions between the side-chains of the two proteins.

Compounds that mimic β-sheets may be identified and/or designed to mimic the binding regions of Ras and Raf. For example, a peptidomimetic compound that mimics the actions of β-sheets may be shown to bind to Ras or Raf in vitro. Such compounds may exhibit antineoplastic or anticancer activity, by blocking the interaction between Ras and Raf.

EXAMPLE 2: Designing of Specific Peptides to Mimic a β-sheet

In some cases such as that described in Example 1 above, a peptide may be purposely designed to mimic a β-strand and/or β-sheet by replacing one or more, and preferably three, of the peptide's amino acids that participate in β-sheet interaction, with Hao or another compound having Formula A. For example, a peptide which mimics a β-strands and/or β-sheets may have the general Formula B, as follows:

$$AA_n - Hao - AA_m$$

wherein AA = amino acid, natural or unnatural, alpha or otherwise, enantomeric or racemic; Hao is Hao or any other compound having the general Formula A as above, and; n = 0 to about 20; m = 0 to about 20; n+m = at least 1.

In addition to the replacement of one or more of the peptide's amino acids, by Hao or another compound of formula A, it may also be desirable to remove amino acids that do not participate in the β-sheet interaction (and in some cases replace those removed amino acids with other chemical groups), thereby creating a specificaly designed peptidomimetic molecule that exhibits desired properties (e.g.. selected β-sheet- like activities, a desired molecular weight; solubility in desired solvents, lipophilicity , bioavailability, long term stability, resistance to enzymatic degradation, etc...)

In other cases, one may wish to select peptides that have specific β-sheet properties (e.g. binding to a specific protein by β-sheet interaction, inhibiting β-sheet interaction between two specific proteins, inhibiting protein

aggregation by β-sheet formation and/or inhibiting β-sheet dimerization of specific proteins) . For example, if one wishes to select a peptide that binds to a certain protein, one could (a) tag that protein with a suitable tag, such as a dye, a fluorophore, a radiolabeled substance, an enzyme, etc. (b) contact the tagged protein in suitable concentration with a one-bead-one-compound combinational library of peptides containing Hao, or other compound of formula A, and (c) then determine the amino acid sequence present in those beads to which the tagged protein has become bound. In this manner one can select from the larger group of previously synthesized peptides, only those which exhibit the desired β-sheet-mediated protein binding activity.

Once a peptide containing Hao or other compounds as described by the general Formula A is identified as being capable of binding to a protein, that particular peptide may be used to identify other compounds which may bind to the particular protein. For example, one can bond, preferably non-covalently bond, the protein to the peptide or compound which mimics β-sheets which comprise a compound having the general formula A to form a complex. This may be achieved by immobilizing the protein and contacting, such as by admixing in a solution, the compound which mimics β-sheets which comprise a compound having the general formula A with the immobilized protein. Then one can contact the test compound with the complex, for example by admixing a solution containing the test compound with a solution containing the complex. After the test compound is introduced into the environment of the complex, the test compounds which have the ability to bind to the protein, for example via a β-sheets protein interaction, will displace the peptide or compound which mimics β-sheets which comprise a compound having the general formula A. Such displacement results in a dissociation of the complex. The dissociation may be determined qualitatively or quantitatively. A qualitative determination that the test compound actually displace the peptide or compound which mimics β-sheets which comprise a compound having the general formula A suggests that test compound may have the ability to interact with a the protein via a β-sheets interaction. A quantitative determination of the degree to which the dissociation of the complex has occurred is then carried out using spectroscopic or other

suitable methods. These quantitative data may then be used as a basis for drawing conclusions regarding the binding affinity of the test compound for the protein.

EXAMPLE 3: Treatments of Huntington's Disease

Glutamine repeats, associated with Huntington's disease and possibly other disorders such as schizophrenia cause proteins to form oligomeric aggregates. These aggregates are thought to have β-sheet structure in which the glutamine side chains hydrogen-bond together, forming a "polar zipper."

Compounds that mimic β-sheets may be identified and/or designed to mimic polyglutamine β-sheet aggregates by employing, for example, methods discussed in the non-limiting Example 2. Such compounds may exhibit the ability to disrupt polyglutamine β-sheet aggregations. On this basis, bioavailable compounds of this invention may have utility in treating or prevention Huntington's Disease.

EXAMPLE 4: Treatments of Alzheimer's Disease

β-Sheet formation plays a key role in Alzheimer's Disease. In Alzheimer's disease, A/β-peptides consisting of 39-43 residues self-assemble to form β-amyloid fibrils in the brain, in a process resembling crystallization. The fibrils have β-sheet structure and exhibit pathogenic properties, including altering signal transduction processes so that neurons lose their processes and enter the pathway for programmed cell death, activating the classical complement pathway, and placing the brain in a state of chronic inflammation. Recently, it is shown that a pentapeptide containing Lys-Leu-Val-Phe-Phe can arrest β-amyloid fibril growth.

It is hypothesized that the interaction of the pentapeptide and the β-amyloid involve β-sheet formation. Compounds that mimic β-sheets may be identified and/or designed to bind to the growing β-amyloid aggregates and block further aggregation. Such compounds may be identified and/or designed, for example, according the methods presented in the non-limiting Example 2. An administration of a therapeutic amount of these compounds to a patient suffering

from Alzheimer's disease may alleviate or at least stop the degenerative process of the disease.

Additionally, compounds that mimic β-sheets which are used to mimic β-sheet interactions in biological systems may provide a tool for further examination of the biological systems. The following examples exemplify how the compounds that mimic β-sheets may be used.

EXAMPLE 5: Studying the Specific Recognition Domains Between Protein G and IgG

In addition to uses of the invention for therapeutic purposes such as those described in Example 2-4 above, it will be appreciated that the invention also has utility in experimental and/or developmental procedures intended to identify compounds that interact with protein through β-sheet interaction, or protein interaction that involves β-sheets.

Protein G is a multidomain cell-surface protein from *Streptococcus*, which binds strongly to immunoglobulin G (IgG). The binding interaction between the 64-residue domain III of the protein G and the Fab portion of IgG includes β-sheet formation.

A peptide which includes a compound of general formula A that may mimic the protein G binding region of the Fab portion of immunoglobulin G is synthesized. Its interaction or non-interaction with domain III of protein G is then studied using NMR or other suitable techniques. The specific recognition domains of the β-sheet interfaces involved in the binding of the Protein G and IgG are identified and characterized. These data are then used to reach a conclusion as to whether, or to what degree, the test peptide mimicked the protein G binding region of the Fab protein.

A Laboratory Synthesis of Hao and Tripeptide A

In synthesizing Hao and Tripeptide A, commercial grade reagents and solvents may be used without further purification. The following is a detailed description of one present preferred method for synthesis of Hao and Tripeptide A.

First, CH_2Cl_2 and THF are dried prior to use by passage through anhydrous Al_2O_3 as described by Grubbs and coworkers. *See*, Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* 1996, *15*, (1518-1520) DMF is dried in an analogous fashion by percolation through 3Å molecular sieves. PS-PEG-PAL-Fmoc resin may be obtained commercially from Applied BioSystems (formerly PE Biosystems), 850 Lincoln Centre Drive, Foster City, CA 94404 U.S.A.

High-resolution mass spectra are obtained by liquid secondary-ion ionization (LSI) of samples in a *m*-nitrobenzyl alcohol matrix bombarded with Cs^+ ions at 25 kV (instrumental variation = 2 mmu). All solution-phase reactions are performed with magnetic stirring; moisture-sensitive solution-phase reactions are carried out in flame- or oven-dried glassware under nitrogen. Solid-phase reactions are performed with mechanical shaking and are, where appropriate, monitored by qualitative ninhydrin tests. Reaction mixtures and product solutions are concentrated by rotary evaporation; where appropriate the residue is further dried using a vacuum pump.

Fmoc*-hydrazine (3). A chilled (0 °C) solution of Fmoc*-Cl (18.72 g, 50.47 mmol) in ether (150 mL) is added to a stirred, ice-cooled solution of anhydrous hydrazine (6.40 mL, 202 mmol) in ether (100 mL) over 2 min. The ice-bath is removed, and the solution is stirred for 3 h. The reaction mixture is transferred to a separatory funnel with ether (50 mL) and wash with water (3 x 150 mL) followed by saturated aqueous NaCl (150 mL). The organic layer is dried over $MgSO_4$, filtered, and concentrated to afford 18.40 g (100%) of Fmoc*-hydrazine (**3**) as a white foamy solid: mp 152-154 °C; IR ($CHCl_3$) 3450, 3352, 1726, 1632 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 7.63 (d, J = 8.0 Hz, 2 H), 7.57 (br s, 2 H), 7.41 (dd, J = 8.0, 1.6 Hz, 2 H), 6.11 (br s, 1 H), 4.43 (d, J = 7.1 Hz, 2 H), 4.17 (t, J = 6.8 Hz, 1 H), 3.78 (br s, 2 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) 158.7, 149.9, 143.7, 138.7, 124.8, 121.8, 119.2, 67.8, 47.2, 34.9, 31.6; HRMS (LSIMS) *m/z* for $C_{23}H_{30}N_2O_2$ $[M]^+$ calcd 366.2307, found 366.2293. Anal. Calcd. for $C_{23}H_{30}N_2O_2$: C, 75.38; H, 8.25; N, 7.64. Found C, 75.53; H, 7.96; N, 7.59.

Hydrazide 5. 2-Methoxy-5-nitrobenzoic acid (4.33 g, 22.0 mmol) is stirred with oxalyl chloride (5.80 mL, 66.0 mmol) and DMF (10 uL) in THF (50 mL) for 1 h. The solvent is removed, and a solution of the resulting acid chloride in CH_2Cl_2 (50 mL) is added over ca. 2 min to a stirred, ice-cold solution of Fmoc*-hydrazine (**3**) and pyridine (1.87 mL, 23.1 mmol) in CH_2Cl_2 (80 mL). The ice-bath is removed and the resulting solution is stirred for 30 min. The reaction mixture is washed with H_2O (150 mL), saturated aqueous $NaHCO_3$ (150 mL), and saturated aqueous NaCl (150 mL), dried over $MgSO_4$, filtered, and concentrated to yield 11.70 g of hydrazide **5** (99%) as a white fluffy solid. An analytical sample is purified by column chromatography on silica gel: mp 126-127 °C; IR ($CHCl_3$) 3410, 1749, 1682, 1616 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 9.42 (br s, 1 H), 9.08 (br s, 1 H), 8.38 (dd, J = 9.1, 3.0 Hz, 1 H), 7.60 (br s, 4 H), 7.41 (d, J = 6.7 Hz, 2 H), 7.22 (br s, 1 H) 7.12 (d, J = 9.2 Hz, 1 H), 4.51 (d, J = 7.0 Hz, 2 H), 4.22 (t, J = 6.8 Hz, 1 H), 4.11 (br s, 3 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) 162.2, 161.6, 156.0, 150.0, 143.5, 142.1, 138.7, 128.9, 128.8, 124.9, 121.9, 120.2, 119.2, 112.0, 68.6, 57.3, 47.0, 34.9, 31.6; HRMS (LSIMS) *m/z* for $C_{31}H_{35}N_3O_6$ $[M]^+$ calcd 545.2526, found 545.2530. Anal. Calcd. for $C_{31}H_{35}N_3O_6$: C, 68.24; H, 6.47; N, 7.70. Found C, 68.13; H, 6.47; N, 7.75.

Amine 6. A 500-mL, three-necked, round-bottomed flask equipped with a stopper, a septum, a magnetic stirring bar, and a three-way stopcock connected to a vacuum line and to a balloon filled with hydrogen is charged with hydrazide **5** (11.70 g, 21.44 mmol), 10% Pd/C (1.20 g), CH_3OH (100 mL) and THF (200 mL). The flask is evacuated and filled with hydrogen (3x), and the reaction mixture is allowed to stir under hydrogen. After 2 h, the flask is evacuated and opened to air, the suspension is filtered through celite, and the celite bed is rinsed thoroughly with ethyl acetate (150 mL). The filtrate is evaporated to dryness to obtain 11.01 g of an off-white solid. Purification by column chromatography (gradient elution with EtOAc:hexanes, 1:20 - 3:1, on a 7.5 cm d x 19 cm h column of silica gel) yield 8.60 g (76% from Fmoc*-Cl) of amine **6** as a white fluffy solid: mp 108-121 °C; IR ($CHCl_3$) 3392, 1736, 1668, 1624 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 9.72 (br s, 1 H), 7.64–7.62 (m, 4 H), 7.55 (dd, J = 2.5, 0.8 Hz, 1 H), 7.41 (d, J = 7.4 Hz, 2 H), 7.18 (br s, 1 H), 6.85-6.81 (m,

2 H), 4.48 (d, J = 7.3 Hz, 2 H), 4.23 (t, J = 7.3 Hz, 1 H), 3.92 (s, 3 H), 3.56 (br s, 2 H), 1.36 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) 164.5, 156.2, 150.8, 149.9, 143.7, 140.7, 138.7, 124.8, 122.0, 120.2, 119.6, 119.2, 118.4, 113.0, 68.5, 56.6, 47.1, 34.9, 31.6; HRMS (LSIMS) *m/z* for $C_{31}H_{37}N_3O_4$ $[M]^+$ calcd 515.2784, found 515.2799. Anal. Calcd. for $C_{31}H_{37}N_3O_4$: C, 72.21; H, 7.23; N, 8.15. Found C, 72.04; H, 7.30; N, 8.15.

Amide 7. To an ice-cooled solution of amine **6** (8.60 g, 16.7 mmol) and pyridine (1.60 mL, 19.8 mmol) in CH_2Cl_2 (150 mL), is added ethyl oxalyl chloride (2.10 mL, 18.8 mmol) in drops over 2 min. After 15 min, the solution is transferred to a separatory funnel with CH_2Cl_2 (50 mL), wash with H_2O (150 mL) and saturated aqueous NaCl solution (150 mL), dried over $MgSO_4$, filtered, and concentrated to yield 10.17 g (99%) of amide **7** as a white solid: mp 222-225 °C; IR ($CHCl_3$) 3390, 1732, 1710, 1672 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 9.74 (br s, 1 H), 9.43 (br s, 1 H), 8.37 (d, J = 8.8 Hz, 1 H), 8.26 (d, J = 2.8 Hz, 1 H), 7.63–7.60 (m, 5 H), 7.41-7.39 (m, 2 H), 7.05 (d, J = 9.2 Hz, 1 H), 4.48 (d, J = 7.2 Hz, 2 H), 4.41 (q, J = 7.2 Hz, 2 H), 4.22 (t, J = 7.0 Hz, 1 H), 4.01 (s, 3 H), 1.40 (t, J = 7.4 Hz, 3 H), 1.35 (s, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$) 163.5, 161.1, 156.0, 154.7, 154.2, 149.9, 143.6, 138.6, 131.0, 125.5, 124.8, 124.2, 121.9, 119.4, 119.2, 112.2, 68.4, 63.7, 56.5, 47.0, 34.8, 31.7, 13.9; HRMS (LSIMS) *m/z* for $C_{35}H_{41}N_3O_7$ $[M]^+$ calcd 615.2944, found 615.2953. Anal. Calcd. for $C_{35}H_{41}N_3O_7$: C, 68.27; H, 6.71; N, 6.82. Found C, 68.53; H, 6.57; N, 6.62.

Fmoc*-Hao (8). To a solution of **7** (10.17 g, 16.52 mmol) in THF:H_2O (300 mL, 4:1) is added 1.00 M NaOH solution (16.55 mL, 16.55 mmol) in a single portion. After 30 min, the solution is passed through a column of Amberlite IR-120(plus) ion-exchange resin (4 cm d x 15 cm h, 100 mL, 1.9 mmol/mL) and concentrated to yield 9.62 g (99%) of Fmoc*-Hao (**8**) as a light tan solid: mp 168-183 °C; IR (KBr) 3700-2400 (br), 3377, 1738, 1693 cm^{-1}; ^{1}H NMR (500 MHz, CD_3SOCD_3) 14.10 (br s, 1 H), 10.81 (s, 1 H), 9.85 (s, 1 H), 9.60 (s, 1 H), 8.17 (d, J = 2.5 Hz, 1 H), 7.88 (dd, J = 9.0, 2.6 Hz, 1 H), 7.77 (s, 2 H), 7.75 (d, J = 8.1 Hz, 2 H), 7.45 (d, J = 8.0 Hz, 2 H), 7.17 (d, J = 9.1 Hz, 1 H), 4.35 (d, J = 7.3 Hz, 2 H), 4.24 (t, J = 7.2 Hz, 1 H), 3.87 (s, 3 H), 1.37 (s, 18 H); ^{13}C NMR (125 MHz, CD_3SOCD_3) 164.9, 162.1, 156.6, 156.2, 153.7, 149.5, 143.8, 138.1, 130.8,

124.6, 124.6, 122.6, 122.1, 121.7, 119.3, 112.3, 66.8, 56.1, 46.6, 34.7, 31.4; HRMS (LSIMS) *m/z* for $C_{33}H_{37}N_3O_7$ $[M]^+$ calcd 587.2631, found 587.2631. Anal. Calcd. for $C_{33}H_{37}N_3O_7$: C, 67.45; H, 6.35; N, 7.15. Found C, 67.67; H, 6.23; N, 6.90.

Boc-Hao. To an ice-cooled solution of pure 5-NH_2-2-MeO-C_6H_3-CONHNHBoc (3.91 g, 13.9 mmol) and Et_3N (2.30 mL, 16.7 mmol) in CH_2Cl_2 (75 mL) is added ethyl oxalyl chloride (1.70 mL, 15.3 mmol) in drops over 1 min. After 15 min, the solution is transferred to a separatory funnel with CH_2Cl_2 (125 mL), wash with H_2O (100 mL) and saturated aqueous NaCl (100 mL), dried over $MgSO_4$ and the solvent is removed to yield 5.25 g (99%) of 5-EtO_2CCONH-2-MeO-C_6H_3-CONHNHBoc as a white solid: mp 184-185 °C; IR ($CHCl_3$) 3388, 1714, 1674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 9.62-9.78 (m, 2 H), 8.37 (dd, *J* = 9.0, 2.8 Hz 1 H), 8.33 (d, *J* = 2.8 Hz, 1 H), 7.47 (br s, 1 H), 7.02 (d, *J* = 9.1 Hz, 1 H), 4.44 (q, *J* = 7.2 Hz, 2 H), 4.00 (s, 3 H), 1.47 (s, 9 H), 1.44 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) 163.2, 161.1, 155.1, 154.7, 154.2, 130.9, 125.3, 124.3, 119.5, 112.1, 81.7, 63.7, 56.5, 28.2, 14.0; HRMS (LSIMS) *m/z* for $C_{17}H_{23}N_3O_7$ $[M]^+$ calcd 381.1536, found 381.1533. Anal. Calcd. for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02. Found C, 53.19; H, 5.76; N, 10.81.

To a solution of 5-EtO_2CCONH-2-MeO-C_6H_3-CONHNHBoc (2.45 g, 6.42 mmol) in THF:H_2O (120 mL, 4:1), is added 1.00 M NaOH solution (6.50 mL, 6.50 mmol) in a single portion. After 30 min, the solution is passed through a column of Amberlite IR-120(plus) ion-exchange resin (2.5 cm d x 10 cm h, 1.9 mmol/mL) and concentrated to yield 2.24 g (99%) of Boc-Hao as a white solid: mp 218-219 °C; IR (KBr) 3600-3400, 3336, 3273, 1720, 1691, 1649 cm^{-1}; ^1H NMR (500 MHz, CD_3SOCD_3) 10.75 (s, 1 H), 9.64 (s, 1 H), 8.93 (s, 1 H), 8.14 (d, *J* = 1.5 Hz, 1 H), 7.83 (dd, *J* = 8.8, 1.8 Hz, 1 H), 7.13 (d, *J* = 9.0 Hz, 1 H), 3.84 (s, 3 H), 1.41 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) 164.8, 162.1, 156.6, 155.2, 153.8, 130.7, 124.6, 122.7, 121.7, 112.3, 79.1, 56.0, 28.1; HRMS (LSIMS) *m/z* for $C_{15}H_{19}N_3O_7$ $[M]^+$ calcd 353.1223, found 353.1230. Anal. Calcd. for $C_{15}H_{19}N_3O_7$: C, 50.99; H, 5.42; N, 11.89. Found C, 50.86; H, 5.39; N, 11.82.

Solid-Phase Synthesis of *i*-PrCO-Phe-Hao-Val-NHBu (9) on PEG-PS-PAL Resin. *Alkylation of the Resin.* PS-PEG-PAL-Fmoc resin (1.071 g, 0.175

mmol/g loading, 0.187 mmol) is shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), wash with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL), and dried under a stream of dry nitrogen. The resin is then shaken with CH_2Cl_2 (10 mL), collidine (149 uL, 1.13 mmol), and 2-nitrobenzenesulfonyl chloride (125 mg, 0.565 mmol) for 3 h. The solution is drained, and the resin is washed with CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen. The resin is then shaken with DMF (10 mL); 1,3,4,6,7,8-hexahydro-1-methyl-2*H*-pyrimido[1,2-*a*]pyrimidine (MTBD, 108 uL, 0.752 mmol) and 1-iodobutane (215 uL, 1.89 mmol) for 8 h. The solution is drained, and the resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen. The resin is then shaken with DMF (10 mL), DBU (140 uL, 0.939 mmol), and β-mercaptoethanol (130 uL, 1.86 mmol) for 3 h. The solution is drained, and this treatment is repeated to ensure complete deprotection. The resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen, to give PS-PEG-PAL(Bu).

Coupling of Val. DCC (0.393 g, 1.90 mmol) is added to a solution of 1.27 g of Fmoc-Val (3.74 mmol) in dichloromethane (47 mL). After 10 min, the resulting white suspension is filtered through a glass frit, the filtrate is concentrated, and the residue (Fmoc-Val anhydride) is suspended in 8:2 CH_2Cl_2:DMF (20 mL). The PS-PEG-PAL(Bu) resin is shaken with half (10 mL) of the suspension for 2 h, the solution is drained, and the resin is shaken with the other half (10 mL) of the suspension for 2 h.[20] The solution is drained, and the resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent),

CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen. The resin is shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), wash with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val.

Coupling of Hao. The PS-PEG-PAL(Bu)-Val resin is shaken with DMF (10 mL), Fmoc*-Hao (440 mg, 0.750 mmol), DCC (154 mg, 0.748 mmol), and HOBt•H_2O (101 mg, 0.66 mmol) for 2 h. The solution is then drained, and the coupling treatment of resin is repeated for an additional 2 h. The solution is drained, and the resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), and ether (2 x 10 mL) and dried under a stream of dry nitrogen. The resin is shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), wash with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao.

Coupling of Phe. The PS-PEG-PAL(Bu)-Val-Hao resin is shaken with DMF (10 mL), Fmoc-Phe (281 mg, 0.725 mmol), DCC (150 mg, 0.725 mmol), and HOBt•H_2O (98 mg, 0.64 mmol) for 2 h. The solution is then drained, and the coupling treatment of resin is repeated for an additional 3 h. The solution is drained, and the resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen. The resin is shaken with three 10 mL-portions of 20% piperidine in DMF (10 min per treatment, draining between treatments), wash with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao-Phe.

Coupling of Isobutyric Acid. The resin is shaken with DMF (10 mL), isobutyric acid (67 uL, 0.72 mmol), DCC (150 mg, 0.73 mmol), and HOBt•H_2O (100 mg, 0.65 mmol) for 1.5 h. The solution is drained, and the resin is washed with DMF (3 x 10 mL), CH_2Cl_2 (3 x 10 mL), methanol (3 x 10 mL), CH_2Cl_2 and methanol (alternately, 3x, with 10 mL-portions of each solvent), CH_2Cl_2 (2 x 10 mL), ether (2 x 10 mL) and dried under a stream of dry nitrogen to give PS-PEG-PAL(Bu)-Val-Hao-Phe-CO-*i*-Pr.

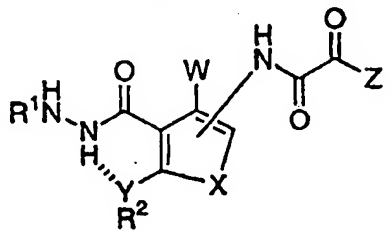
Cleavage and Purification. The PS-PEG-PAL(Bu)-Val-Hao-Phe-CO-*i*-Pr resin (0.969 g) is allowed to stand with 10% TFA in CH_2Cl_2 (47 mL) for 1 h without agitation. The solution is filtered and concentrated, and the resultant oil is dissolved in CH_2Cl_2 (100 mL) and wash with saturated aqueous K_2CO_3 (100 mL). The organic layer is removed, dried over $MgSO_4$, filtered, and concentrated. The resultant solid (71 mg) is dissolved in hot MeOH (3 mL) and allowed to precipitate. The precipitate is collected in two crops by filtration and dried under vacuum to give 46 mg (39% yield, 46% corrected for resin losses) of *i*-PrCO-Phe-Hao-Val-NHBu (Tripeptide A) as a white solid: mp 152 °C dec. IR (KBr) 3442, 1641; ^{1}H NMR ($CDCl_3$, 400 MHz) 11.73 (br s, 1 H), 11.18 (d, *J* = 7.8 Hz, 1 H), 10.70 (s, 1 H), 8.65 (d, *J* = 2.5 Hz, 1 H), 8.49 (dd, *J* = 9.0, 2.5 Hz, 1 H), 8.41 (d, *J* = 9.4 Hz, 1 H), 7.91 (br. s, 1 H), 7.18-7.25 (m, 3 H), 7.12 (d, *J* = 6.6 Hz, 2 H), 7.04 (d, *J* = 9.2 Hz, 1 H), 6.34 (d, *J* = 7.2 Hz, 1 H), 5.38 (appar. quartet, *J* = 6.8 Hz, 1 H), 4.61 (appar. t, *J* = 8.5 Hz, 1 H), 4.04 (s, 3 H), 3.35 (appar. sextet, *J* = 6.8 Hz, 1 H), 3.23 (dd, *J* = 13.7, 6.3 Hz, 1 H), 3.08-3.16 (m, 2 H), 2.40 (appar. septet, *J* = 6.9 Hz, 1 H), 2.17 (septet, *J* = 7.0 Hz, 1 H), 1.51 (appar. pentet, *J* = 7.3 Hz, 2 H), 1.35 (sextet, *J* = 7.4 Hz, 2 H), 1.14 (d, *J* = 6.9 Hz, 3 H), 1.13 (d, *J* = 6.8 Hz, 3 H), 1.06 (d, *J* = 6.6 Hz, 3 H), 1.05 (d, *J* = 6.7 Hz, 3 H), 0.90 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) 176.7, 169.8, 165.1, 159.8, 158.8, 157.8, 154.2, 135.5, 131.6, 129.5, 128.4, 127.1, 126.4, 124.5, 119.1, 111.8, 59.2, 56.6, 51.4, 39.9, 39.2, 35.5, 31.9, 31.6, 20.2, 19.7, 19.2, 19.0, 13.8; HRMS m/z for $C_{32}H_{44}N_6O_7Na$ $[M+Na]^+$ Calcd. 647.3169, found 647.3195. A sample of *i*-PrCO-Phe-Hao-Val-NHBu (which is marked by reference numeral 9 on Figure 5 and is referred to herebelow as "Tripeptide A), prepared by solid-phase synthesis on

indole resin as described above, is subjected to elemental analysis: Anal. Calcd. for $C_{32}H_{44}N_6O_7$: C, 61.52; H, 7.10; N, 13.45. Found C, 61.39; H, 7.12; N, 13.28.

It is to be appreciated that the invention has been described hereabove with reference to certain embodiments and examples, but that no effort has been made to exhaustively describe all possible embodiments and examples of the invention. Those of skill in the art will recognize that various changes, additions, modifications, substitutions and deletions may be made to the specific examples and embodiments described hereabove, without departing from the intended spirit and scope of this invention.

CLAIMS

1. A composition have the general formula :



wherein,

R^1 = H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

R^2 = H, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

W = H, F or the NH-CO-CO-Z group shown,

X = O, S, NR^3, CR^4=N, N=CR^4, CR^4 =CR^5;

R^3 is H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

R^4 and R^5 are each selected from H, alkyl, halogen, nitro, carboxyl, amino, alkyl or aryl sulfone, alkyl or aryl sulfoxide, sulfonic acid, sulfonate salt or sulfonamide, and wherein R^4 and R^5 may be combined to form a ring structure;

Y = O, S, or YR^2 as a group may be a halogen;

Z = OR^6 or NR^7R^8, wherein R^6, R^7 and R^8 are each selected from H, acyl, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide.

2. A peptide incorporating composition according to Claim 1.

3. A peptide incorporating composition according to Claim 1, wherein the composition induces the peptide to fold into β-sheets.

4. A protein incorporating a composition according to Claim 1.

5. A peptidomimetic compound incorporating a composition according to Claim 1.

6. A composition according to Claim 1 combined with an agent to cause that agent to mimic β-strands.

7. A compound according to Claim 1 combined with an agent to cause that agent to block β-sheet dimerization of proteins.

8. A compound according to Claim 1 combined with an agent to cause that agent to block protein-protein β-sheet interactions.

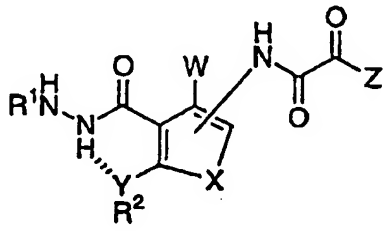
9. A compound according to Claim 1 combined with an agent to cause that agent to interact with a protein by β-sheet formation.

10. A tripeptide compound according to Claim 1 comprising *i*-PrCO-Phe-Hao-Val-NHBu.

11. A preparation comprising a composition according to Claim 1 in a pharmaceutically acceptable carrier.

12. A method of causing dimerization of a compound that is capable of dimerizing due to β-sheet interactions, said method comprising the step of:

combining the compound with a chemical entity having the general formula:



wherein;

R^1 = H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

R^2 = H, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

W = H, F or the NH-CO-CO-Z group shown,

X = O, S, NR^3, CR^4=N, N=CR^4, CR^4 =CR^5;

R^3 is H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

R^4 and R^5 are each selected from H, alkyl, halogen, nitro, carboxyl, amino, alkyl or aryl sulfone, alkyl or aryl sulfoxide, sulfonic acid, sulfonate salt or sulfonamide, and wherein R^4 and R^5 may be combined to form a ring structure;

Y = O, S, or YR^2 as a group may be a halogen;

Z = OR^6 or NR^7R^8, wherein R^6, R^7 and R^8 are each selected from H, acyl, alkyl or aryl with up to 20 carbon atoms, which may be straight or

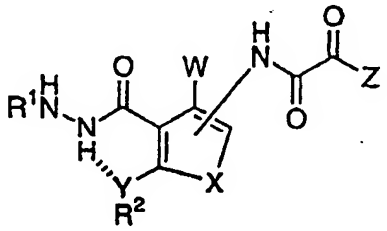
branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide.

13. A method according to Claim 9 wherein the compound is a peptide.

14. A method according to Claim 9 wherein the compound is a protein.

15. A method according to Claim 9 wherein the compound is a peptidomimetic compound.

16. A method of treating a disease or disorder in a human or animal patient, said method comprising the step of administering to the patient a therapeutically effective amount of a compound having the formula:



wherein;

R^1 = H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

R^2 = H, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

W = H, F or the NH-CO-CO-Z group shown,

X = O, S, NR^3, CR^4=N, N=CR^4, CR^4 =CR^5;

R^3 is H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

R^4 and R^5 are each selected from H, alkyl, halogen, nitro, carboxyl, amino, alkyl or aryl sulfone, alkyl or aryl sulfoxide, sulfonic acid, sulfonate salt or sulfonamide, and wherein R^4 and R^5 may be combined to form a ring structure;

Y = O, S, or YR^2 as a group may be a halogen;

Z = OR^6 or NR^7R^8, wherein R^6, R^7 and R^8 are each selected from H, acyl, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide.

or a pharmaceutically acceptable salt thereof.

17. A method according to Claim 13 wherein the disease or disorder being treated is a cancer and the compound mimics a β-sheet which binds with a Ras oncoprotein.

18. A method according to Claim 13 wherein the disease or disorder being treated is cancer and the compound mimics a β-sheet which binds to the Ras-binding domain of serine/kinase c-Raf1 (Raf).

19. A method according to Claim 13 wherein the disease or disorder being treated is a neurodegenerative disease wherein proteins form oligomeric aggregates and wherein the compound comprises mimics a β-sheet which disrupts the formation of such oligomeric aggregates.

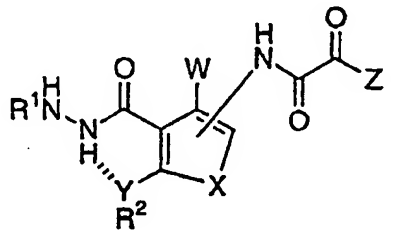
20. A method according to Claim 16 wherein the compound mimics a polyglutamine β-sheet aggregate.

21. A method according to Claim 16 wherein the disease or disorder is Huntington's Disease or schizophrenia.

22. A method according to Claim 13 wherein the disease or disorder is Alzheimer's Disease and wherein the compound comprises a compound that mimic β-sheet which binds to β-amaloyd aggregates and block β-amaloyd fibril growth.

23. A method for identifying compounds which participate in β-sheet interaction with a protein, the method comprises the steps of:

a) providing a protein, a test compound, and a compound which mimics β-sheets which comprise a compound having the general formula:



wherein;

R^1 = H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

R^2 = H, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

W = H, F or the NH-CO-CO-Z group shown,

X = O, S, NR^3, CR^4=N, N=CR^4, CR^4 =CR^5;

R^3 is H, acyl or alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral;

R^4 and R^5 are each selected from H, alkyl, halogen, nitro, carboxyl, amino, alkyl or aryl sulfone, alkyl or aryl sulfoxide, sulfonic acid,

sulfonate salt or sulfonamide, and wherein R^4 and R^5 may be combined to form a ring structure;

Y = O, S, or YR^2 as a group may be a halogen;

Z = OR^6 or NR^7R^8, wherein R^6, R^7 and R^8 are each selected from H, acyl, alkyl or aryl with up to 20 carbon atoms, which may be straight or branched, cyclic or acyclic, chiral or achiral, or an amino acid or peptide;

b) non-covalently bond the protein to the compound which mimics β-sheets which comprise a compound having the general formula A to form a complex;

c) contact the test compound with the complex; and

d) determine the dissociation of the complex.

24. A method according to Claim 23 wherein the step of non-covalently bonding the protein is performed by ii) immobilizing the protein and ii) contacting the immobilized protein with the compound which mimics β-sheets.

25. A method according to Claim 23 wherein step of contacting the test compound is performed by admixing a solution containing the test compound with a solution containing the complex.

26. A method according to Claim 23 wherein step of determining the dissociation of the complex is a quantitative determination.

27. In a peptide synthesis wherein amino acids are added sequentially to a growing peptide chain, a method of attaching one amino acid to another amino acid or peptide chain, said method comprising the steps of:

(A) attaching to the *N* terminus of the amino acid to be added a protecting group comprising Fmoc*;

(B) causing the amino acid to form a peptide linkage with the other amino acid or peptide chain such that the protecting group that had been attached to the amino acid in Step A is at the *N* terminus of the growing peptide chain.

28. A method according to Claim 27 further comprising the steps of:

(C) detaching said protecting group from the *N* terminus of the growing peptide chain.

29. A method according to Claim 28 further comprising the step of:

(D) attaching to the end terminus of another amino acid a protecting group comprising Fmoc*;

and,

(E) causing the amino acid of Step D to form a peptide linkage with the N terminus of the peptide chain such that the protecting group that had been attached to the amino acid in Step D is at the *N* terminus of the growing peptide chain.

30. An *N*–terminally protected amino acid having the formula:

P-AA

wherein AA is an amino acid and P is Fmoc*

31. An *N*–terminally protected peptide having the formula:

$P\text{-}(AA)_n$

wherein AA is an amino acid, P is Fmoc* and n is 2 or more.

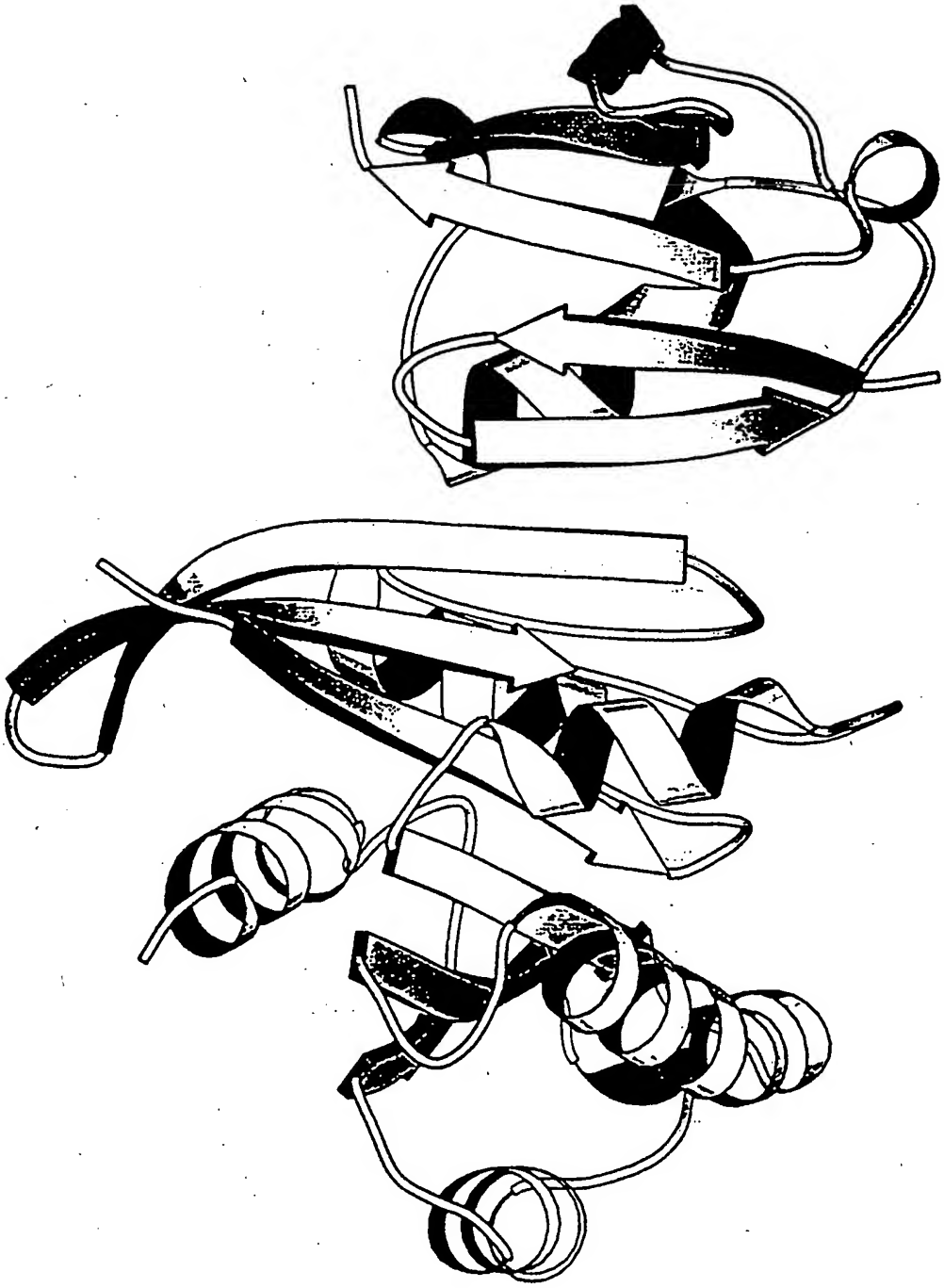


FIG. 1

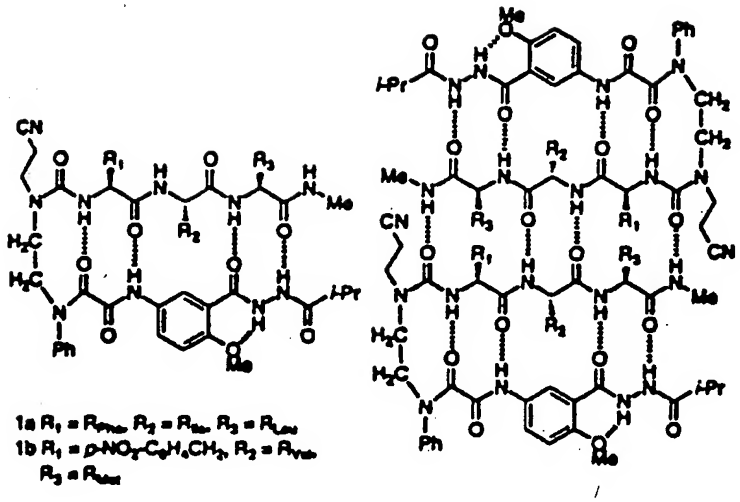


FIG. 2

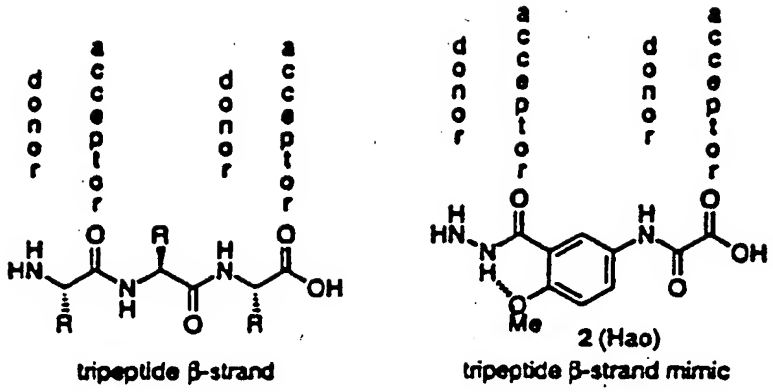

donor
acceptor
donor
acceptor
tripeptide β-strand
donor
acceptor
donor
acceptor
2 (Hao)
tripeptide β-strand mimic

FIG. 3

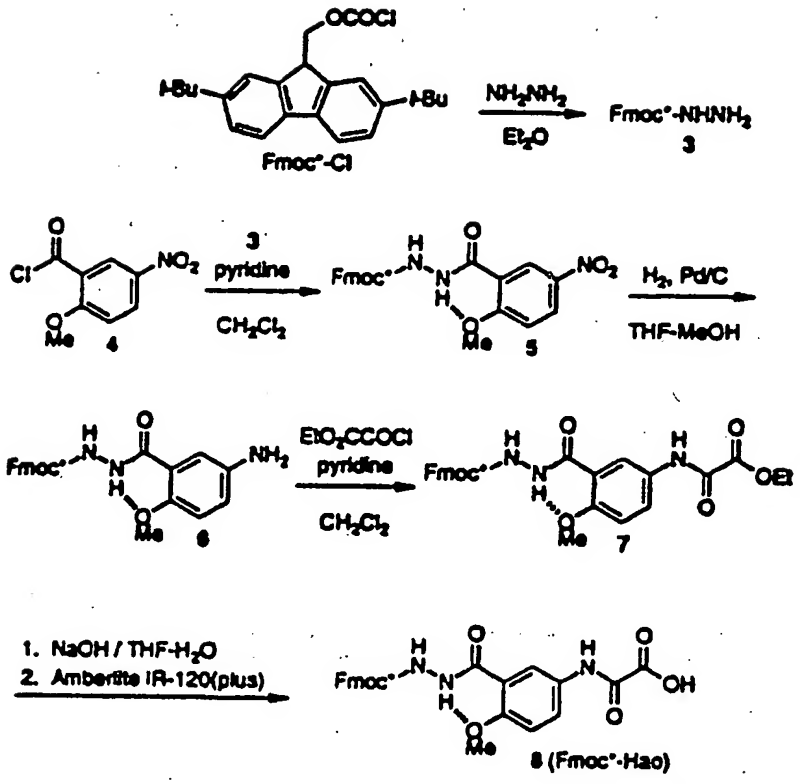

OCOCl
t-Bu
t-Bu
Fmoc*-Cl
NH2NH2
Et2O
Fmoc*-NHNH2
3
Cl
NO2
O
Me
4
3
pyridine
CH2Cl2
Fmoc*
N
H
O
Me
5
NO2
H2, Pd/C
THF-MeOH
Fmoc*
NH2
6
EtO2CCOCl
pyridine
CH2Cl2
7
OEt
1. NaOH / THF-H2O
2. Amberlite IR-120(plus)
OH
8 (Fmoc*-Hao)

FIG. 4

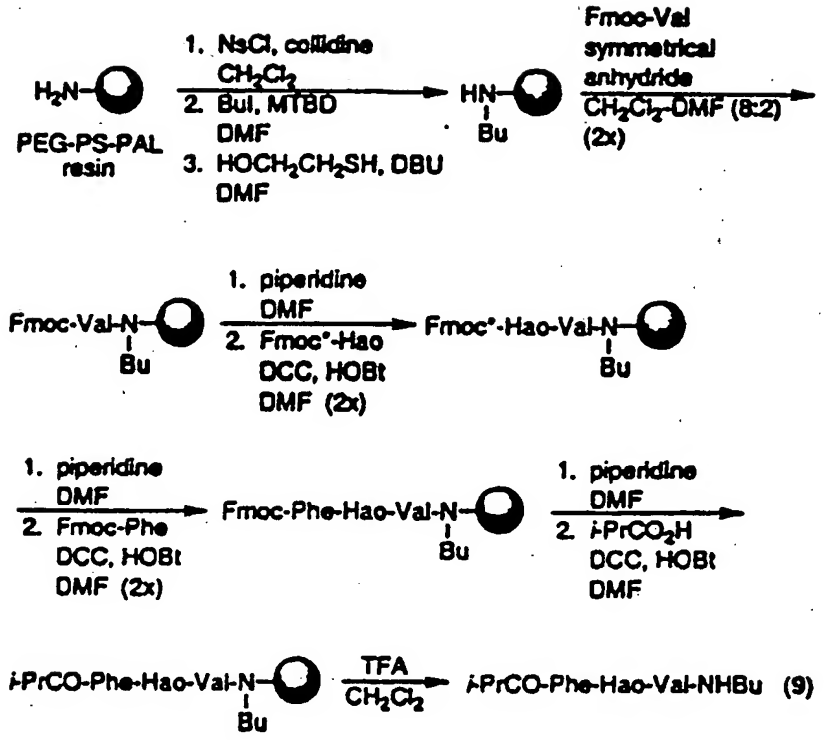

H2N
PEG-PS-PAL resin
1. NsCl, collidine CH2Cl2
2. BuI, MTBD DMF
3. HOCH2CH2SH, DBU DMF
HN
Bu
Fmoc-Val symmetrical anhydride
CH2Cl2-DMF (8:2)
(2x)
Fmoc-Val-N
Bu
1. piperidine DMF
2. Fmoc*-Hao DCC, HOBt DMF (2x)
Fmoc*-Hao-Val-N
Bu
1. piperidine DMF
2. Fmoc-Phe DCC, HOBt DMF (2x)
Fmoc-Phe-Hao-Val-N
Bu
1. piperidine DMF
2. i-PrCO2H DCC, HOBt DMF
i-PrCO-Phe-Hao-Val-N
Bu
TFA
CH2Cl2
i-PrCO-Phe-Hao-Val-NHBu (9)

FIG. 5

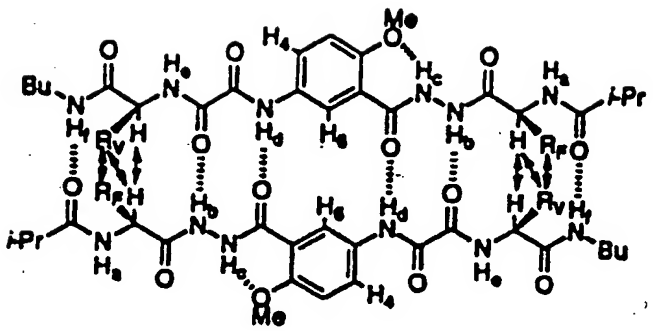


FIG. 6

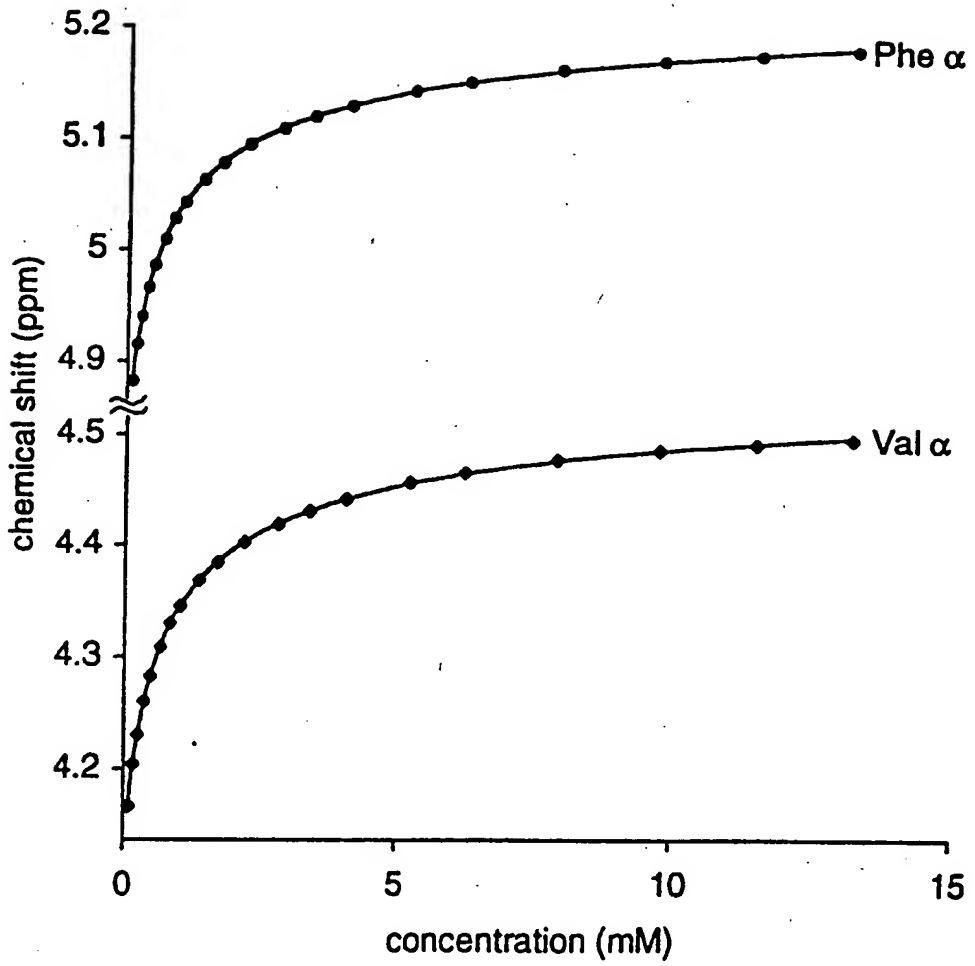

5.2
5.1
5
4.9
4.5
4.4
4.3
4.2
chemical shift (ppm)
Phe α
Val α
0
5
10
15
concentration (mM)

FIG. 7

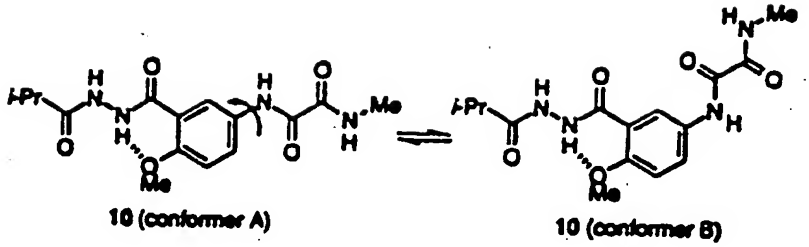
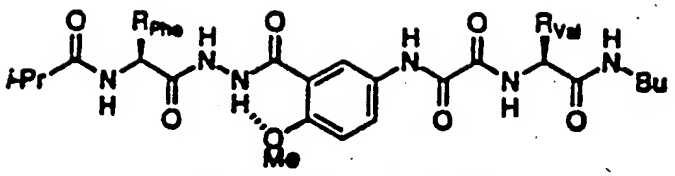


FIG. 8



9 (*i*-PrCO-Phe-Hao-Val-NHBu)

FIG. 9

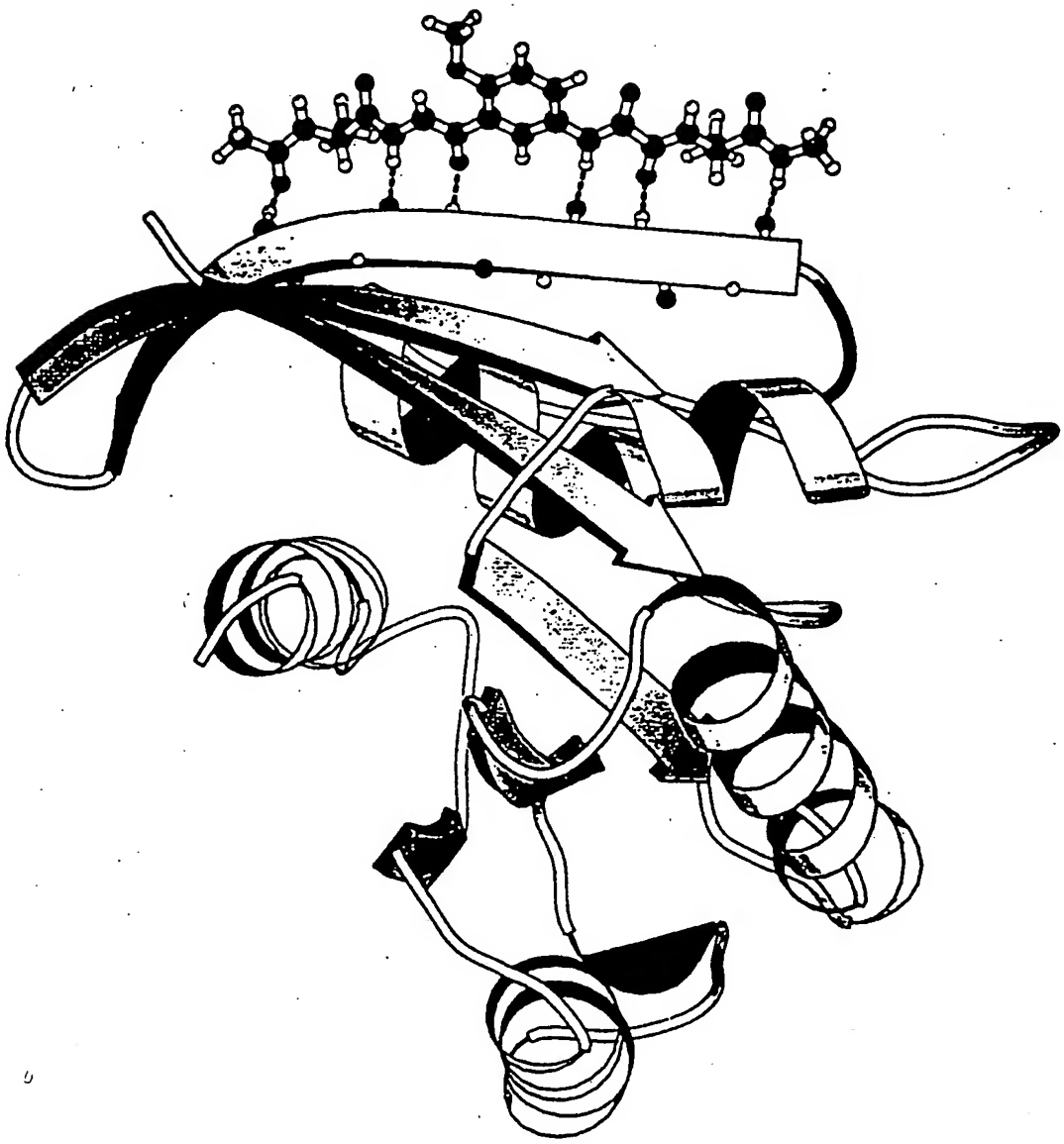


FIG. 10

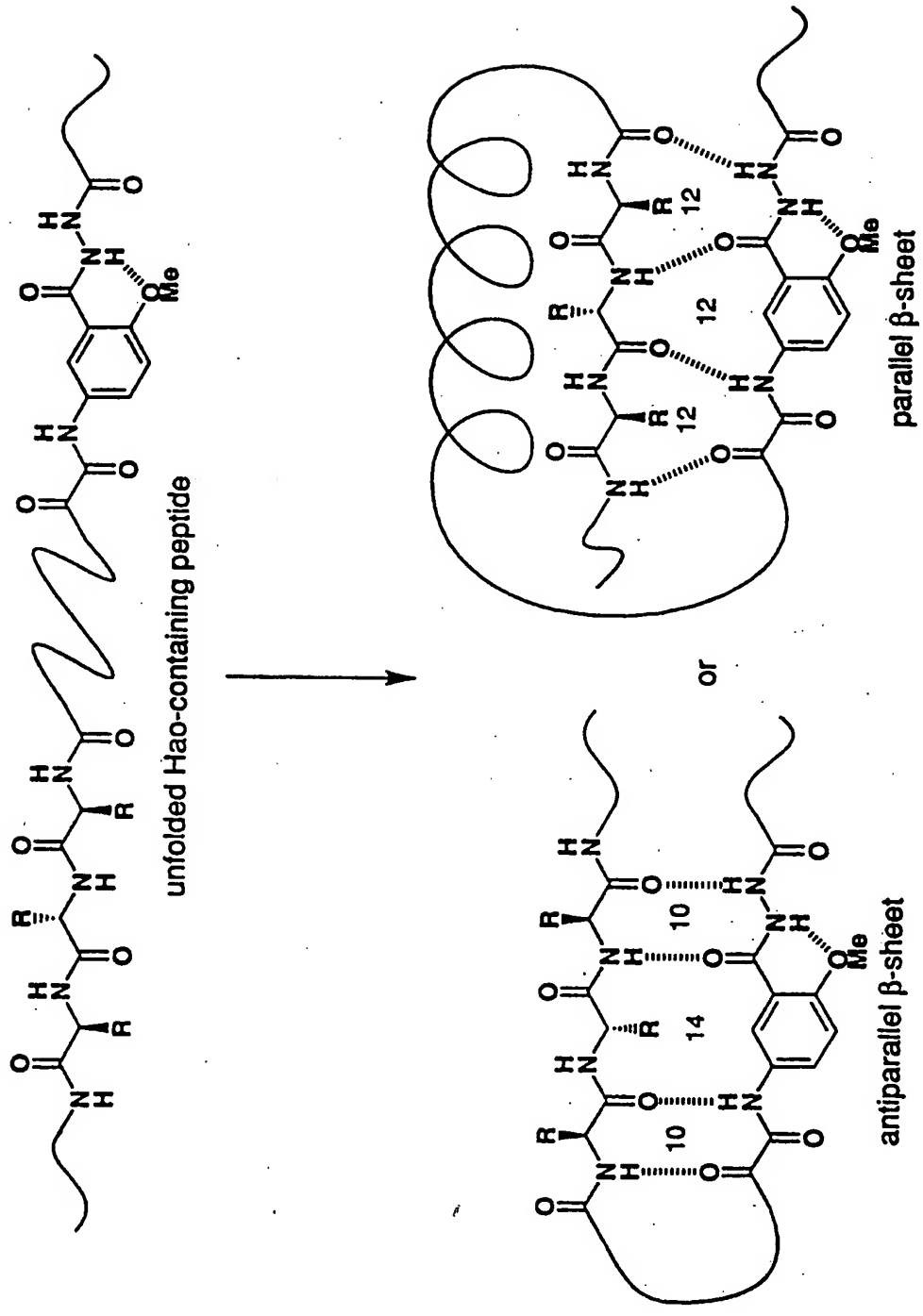
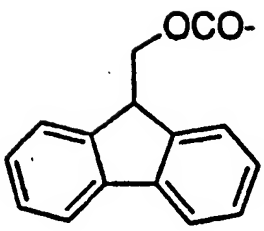
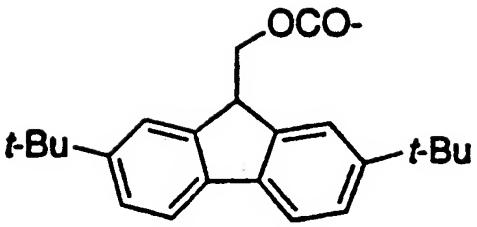

unfolded Hao-containing peptide
parallel β-sheet
or
antiparallel β-sheet

FIG. 11



Fmoc



Fmoc*

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/23049

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C07K 5/02
US CL :530/331
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/331

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 6,020,331 A (KAHN) 01 February 2000, see entire document.	1-31
A	US 5,618,914 A (KAHN) 08 April 1997, see entire document.	1-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 OCTOBER 2000	Date of mailing of the international search report 15 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID LUKTON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★